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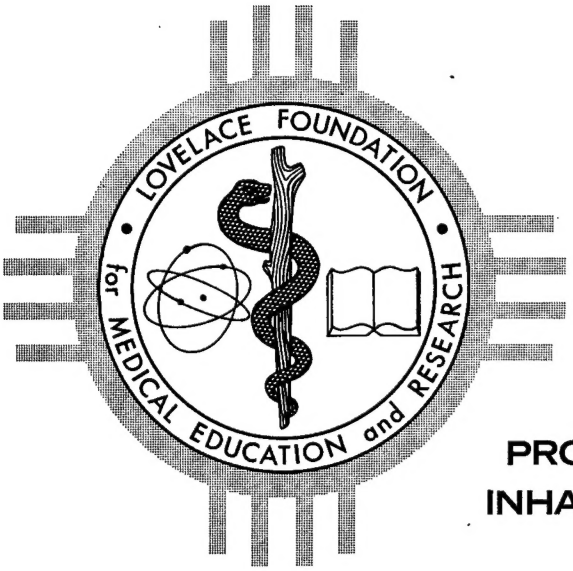

PROCEDURES AND EQUIPMENT USED IN INHALATION STUDIES ON SMALL ANIMALS

by

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Albuquerque, New Mexico

September 1963

ATOMIC ENERGY COMMISSION-
LOVELACE FOUNDATION
FISSION PRODUCT INHALATION PROJECT

LF-11
Biology and Medicine
TID-4500 (22nd Ed.)

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Submitted as a
Technical Progress Report
to
The Division of Biology and Medicine
United States Atomic Energy Commission
on
Contract No. AT(29-2)-1013
September 1963

From the Department of Radiobiology
Lovelace Foundation for Medical Education and Research
Albuquerque, New Mexico

ABSTRACT

Procedures and equipment used in exposing small animals to aerosols of fission products are described. The exposure chamber with attached plethysmographs for monitoring the respiratory patterns is shown diagrammatically and the operational plan is set forth. Construction criteria for the plethysmographs are also included in detail. The descriptions concerning the animals include specifications on metabolism units and cleaning procedures, whole body counting and somewhat detailed sacrifice and necrotomy procedures.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the advice and actual participation in designing the exposure apparatus (Dr. T. T. Mercer) and the transducer and recording systems (Mr. R. M. Goodrich). We also wish to thank Dr. B. B. Boecker for his consultation on many aspects of the entire small animal program.

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INTRODUCTION

The assessment of potential hazards that might be associated with inhaling radioactive materials requires that a quantitative relationship be established between the parameters characterizing the environmental challenge and the biological consequences of exposure thereto. Appropriate and adequate studies must determine: (a) the concentration, size and solubility of the product in the air inhaled; (b) the respiratory factors that govern the amount of aerosol reaching the airways; (c) the site and total portion of the material initially deposited; and (d) the biological residence time as governed by the kinetics of tissue distribution and excretion. When these parameters have been quantitated on a variety of species, an extrapolation to the human may be attempted.

"Mass exposure" techniques have been used in the past, employing an adapted "pickle jar" [1, 2, 3] to hold several animals at a time and a Dautreband aerosolizer [4] to generate the radioactive atmosphere. These procedures are adequate for exposing large numbers of animals in a short time and for providing whole animals, tissues and organs for distribution and excretion studies. However, sufficiently accurate information has not been forthcoming on either the concentrations of aerosols to which each animal was exposed or on the individual respiratory characteristics during the exposure period.

These facts prompted the initiation of an effort to develop appropriate exposure equipment. The purpose of this communication is first, to describe an apparatus which, with modifications, allows pertinent information to be obtained on each individual small animal exposed and

secondly, to set forth the pre- and post-exposure procedure adopted for obtaining required quantitative data regarding respiration, deposition, distribution and excretion.

EXPOSURE APPARATUS

An exposure apparatus was designed to accommodate five small animals and sufficient aerosol characterization equipment to determine particle size distribution and air concentration. The central chamber into which the aerosol was introduced was hexagonal in design, with an animal's nose protruding into each of five sides. The sixth side was used for sampling certain of the aerosol characteristics. Each animal was housed in a plethysmograph which could monitor breathing movement due to volume change, posteriorally from the neck. Only the neck and head were not included in the plethysmograph, an obvious restriction necessitated by the manner of introducing the aerosol. This entire exposure apparatus, plus the aerosolization equipment, was housed in a dry box operating at slight negative pressure. The details of this exposure setup follow:

Overall Construction. Figure 1 will give the reader a sufficient perspective of size and physical placement of the component parts. Except where otherwise obvious all parts are constructed from lucite plastic, either molded or machined. The drawing is presented in an exploded version to more clearly define the individual components while still indicating their positioning in the assembled state.

Aerosol enters the exposure chamber at the center bottom where it is randomly mixed with diluent air through a series of ports around the aerosol inlet. With the plethysmograph in place, aerosol is drawn past each animal's nose and upward through the membrane filter positioned directly above. Thus, a rather precise measurement is obtained of the radioactivity in the atmosphere to which each animal was exposed. Also, knowing the flow rate and time of exposure, a value for air concentration

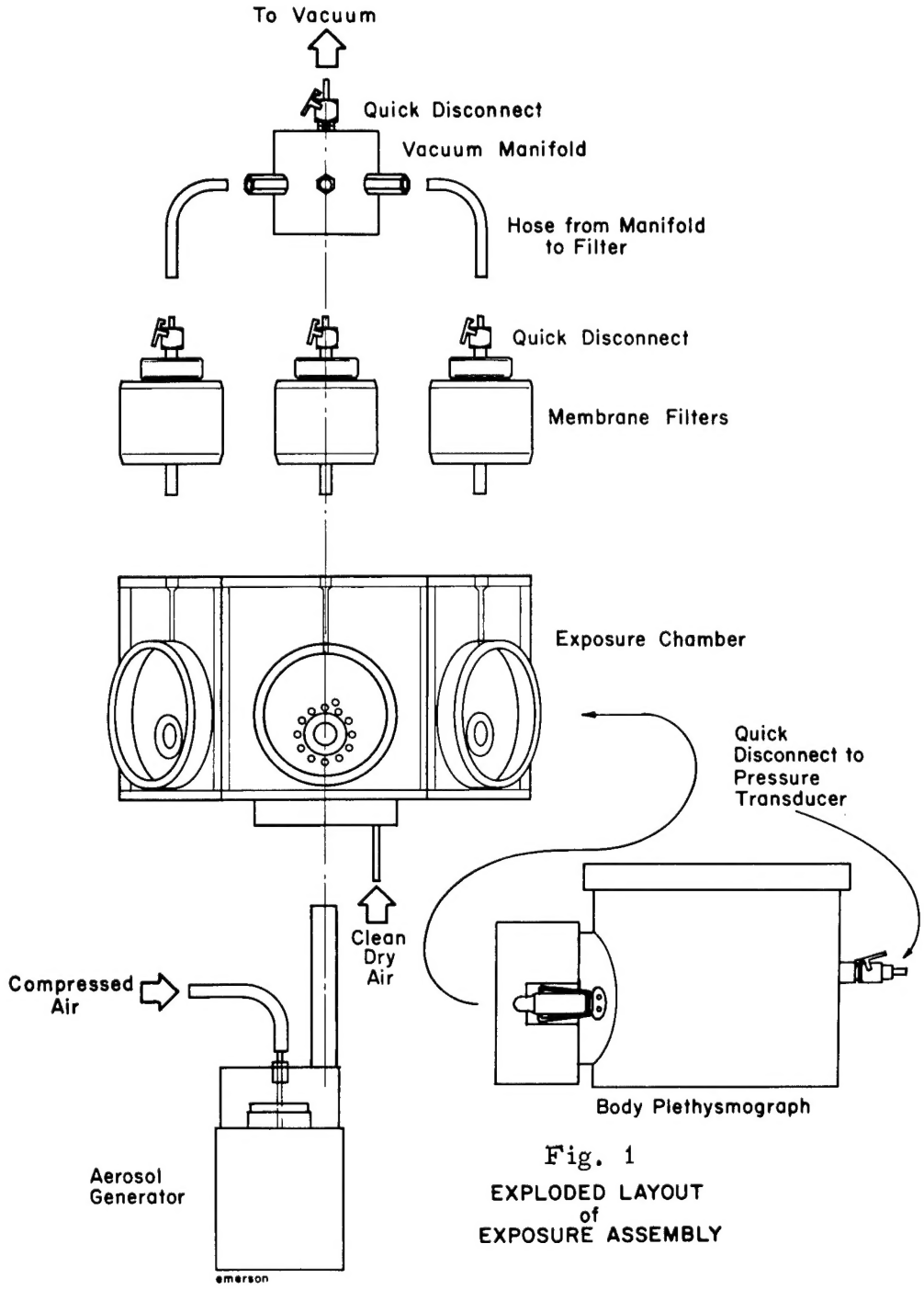


Fig. 1
EXPLODED LAYOUT
of
EXPOSURE ASSEMBLY

can be calculated. These values can then be compared to that obtained from the central filter used to remove the remainder of the aerosol, thus yielding a comparison of air concentration at various positions around the chamber.

Although this exposure apparatus was used quite extensively it did prove to have many poor design features. The method of connecting the plethysmographs to the main chamber was clumsy and often required a great deal of force. The plethysmograph lids were very sensitive after repeated usage and had to be scanned carefully to render them "air-tight". Although, with certain modifications, this could be used for future low-radioactivity level exposures, it was desirable to design and construct a new apparatus which could also be utilized for highly radioactive aerosols. One of the obvious alteration requirements is a much smaller exposure chamber.

1. Exposure Chamber - New Design Criteria

The new exposure apparatus, presently under construction, will employ approximately the same type of hexagonal configuration. However, there will be certain features required for the higher levels of exposure, these changes being mostly dictated by the greatly increased shielding requirements. The entire apparatus will also be on a turntable so that access to each plethysmograph will be from one convenient location. The dry box enclosure will also incorporate changes to make easier the handling of the shielded aerosol generators once they have been "charged" with the proper radioactive solutions.

The Plethysmographs

1. The Pneumatic Collars

One of the primary requirements for the plethysmograph is a tight seal around the animal's head or neck. This is necessary to keep aerosol out of the plethysmograph and, more important, to guarantee that all recorded changes in the pressure within are directly proportional to the breathing volume changes. Any leaks around this seal during an

exposure would render the calibration data useless. To accomplish such a seal, a pneumatic collar was made which resembled that developed at Linde Corporation in Tonawanda, New York[5]. The plethysmographs were also designed, in principle, after that described by Gottlieb[5]. Construction of the collars and their molds, plus their assemblage and usage is described in detail in Appendix I. The description will give the interested reader the stepwise procedure as used in this Laboratory.

2. Proper Plethysmograph Volume

The volume of the plethysmograph must be chosen so that increases in pressure due to inspiration will not exert a reverse force (overpressure) upon the animal which may, in turn, be large enough to alter the respiratory pattern. We also desired to have chambers of such volume that they could be adapted for use with all three small animal species of interest to us; i.e., mice, rats and guinea pigs. If the same calibration were to be utilized for all species, it would obviously require a plethysmograph large enough to accommodate the largest species, with an additional static volume being added to compensate for the small volumes of the mouse and rat. However, we have not been concerned with this and have recalibrated for each species being used.

Calibration of the system is accomplished by placing a volume of water (in a plastic bag), comparable to the animal volume, into the plethysmograph and connecting it to an automatic pipetting system. With the stroke set at the approximate tidal volume expected, the chart is calibrated by overall trace height versus tidal volume.

It has been found that over a static volume range in the plethysmograph of 100 to 500 cc., and using a constant stroke volume of both 1 and 2 cc.'s, the maximum change in calibration characteristics is not more than 15%, within the experimental limits of measurement. A more detailed description of the plethysmograph's operational characteristics along with some basic data on the three small animal species, will be reported at a later date.

The proper plethysmograph volume may be quite simply calculated and is done so, for our particular case, in Appendix II. *

Aerosolization and Characterization. The aerosol generators used have gone through several modifications and the one presently in use is being documented [6]. This is also true of the instrumentation and apparatus used for characterization of the atmosphere [7, 8].

ANIMAL TREATMENT

Pre-Exposure Preparation. Approximately 15 minutes prior to exposure each animal is injected intraperitoneally with an anesthetic solution containing chloral hydrate, magnesium sulfate and pentobarbital sodium (in proportions 4.6 : 2.3 : 1.0 by weight). The dosage used is 20-22 mg pentobarbital sodium per kg body weight; the solution volume is approximately three milliliters per kg. This anesthetic, in these proportions and quantities, provides an adequate depth of anesthesia in 10 to 15 minutes, which has a duration of from 30 to 45 minutes. Under these circumstances the respiratory rate and depth of breathing do not appear to be affected greatly from the normal.

Following anesthetization, the animals' necks are shaved (with clippers) and a water-soluble jelly (Aquaresin, Glyco Chemicals Div. of the Chas. L. Huisking and Co., Inc.) is applied to the shaved area. The animal is then inserted into the plethysmograph as described in Appendix I.

Post-Exposure Techniques. After the exposure is complete and the chamber has been flushed with clean air, the plethysmographs are detached and the lids removed. The animals are then taken from the plethysmograph by handling only the clean areas (posterior from the

*The procedure for doing this particular calculation was suggested to us by Dr. C. S. White and the "overpressure" criterion (1 cm. water) used was suggested by Dr. U. C. Luft, both of Lovelace Foundation.

neck) and the entire head is washed. This is accomplished with gauze sponges using a fairly concentrated decontamination solution (Radiacwash, Atomlab, Inc.). The head is then rinsed with running water and is dried thoroughly with gauze sponges. This procedure allows a containment of most of the radioactive waste on the sponges.

If the animal is to be used in initial body deposition study, it is sacrificed upon removal from the plethysmograph. If it is to be part of a distribution and excretion study, it is merely transferred to a metabolism cage after the head is dried and whole body radioactivity is determined.

RESPIRATION MEASUREMENT

Transducer System. The pressure changes due to breathing are converted to an electrical signal by means of pressure transducers. These transducers, Model PM 197, are manufactured by Statham Instruments, Inc., Los Angeles, California, and have a pressure sensitivity range of ±0.01 pounds per square inch (psi). They are resistive, balanced, complete unbonded strain gage bridges and are practically insensitive to temperature changes with zero shifts typically less than 0.01% of full scale per degree Fahrenheit. The total differential error is less than 0.003 inch of water; combined non-linearity and hysteresis is less than ±0.5% of full scale. The nominal bridge resistance is 350 ohms.

The Electrical System. The transducer operates on a carrier frequency of 2000 cycles per second (cps) supplied by a Brush Universal Amplifier, Model RD 5612 00. The signal is then recorded on chart paper in a Brush Dual Oscillograph, Model RD 2622 00.

Respiration Data Analysis, Present and Future. The recording paper to date has been analyzed manually, with the amplitude of each breath being measured with a rule over a given time period of recording. The minute-volume is then obtained from the calibration data by simple translation of amplitude into volume. The breathing rate is obtained by counting the

number of breaths taken per unit of time.

Analysis by this method is extremely tedious and time consuming and will, in the future, be replaced by an electronic system. This will be a device to convert the analog signal from the breathing phenomenon into a digital signal which will then read out the data on mechanical registers. One register will record total volume breathed over the period of exposure and the other will record the total breaths taken, for each of the five animals being exposed. Simple arithmetic will yield the average minute and tidal volumes.

"METABOLISM" STUDIES

Philosophy and General Importance. As stated under Introduction, there are two general schemes to be followed with the animals after inhalation exposure. Immediate sacrifice will yield respiratory tract and whole body deposition data and serial sacrifices accompanied by urine and feces collections will yield data on nuclide retention characteristics. Data from this latter procedure can be used (when extrapolated properly to man) to arrive at suitable bioassay methods to determine the body burden of an exposed individual in cases where whole body counting is not feasible. Also, along with the data from individual tissue analyses, the excretion data can be very useful in determining the intricate pathways of metabolism of a material. In experimentation, excretion data also serve a very useful purpose in helping to determine the total recovery (balance) of a radionuclide from a given animal.

In this Section, the methods of obtaining the excreta in a form suitable for a radioactivity determination will be discussed.

The Collection Setup

1. Cage Requirements

The cages were selected to be of uniform size regardless of the small animal species to be studied. They either house one guinea pig, two rats, or at least four mice per cage and a composite collection of

urine and feces samples is made for the latter two. The cage has only enough ventilation for a healthy amount of air turnover but is sufficiently enclosed to avoid any harmful drafts (air currents). The final design has solid stainless steel sheeting on three sides and 1/2" (1/3" for mice) mesh wire on the bottom and the front. The removable lids are of solid stainless steel plate, affixed with a hole for a water spout. The overall cage dimensions are 7"H x 10"D x 8"W.

2. Collection Trays

The collection trays were designed to be fitted with hardware cloth which rests approximately 1-1/4 inches below the bottom of the cage and has dimensions equal to that of the cage bottom. The tray is beveled from all four sides to a 1/2" hole at the center. This hole is designed to hold a small wad of cotton so as to prevent any feces which accidentally pass the wire mesh screen from falling into the urine container below. The cotton allows the easy passage of urine and water, and also serves to guide the liquid into the urine container.

The entire cage and tray assembly is placed upon a wooden stand of such a height to accommodate the urine collection container. Each animal or set of animals has two cage units, one directly below (or above) the other. Each day the animal(s) is (are) moved to the unused cage while the other is being cleaned and readied for receiving the animal(s) the next day. Thus, the animals should only contaminate their own cage setups and the likelihood of other cross-contamination is considerably reduced. This is an inefficient use of metabolism units and, in the future, it may be desirable to change the practice of having half of them unused for the most part of each twenty-four hour period.

Figure 2 is a detailed drawing of a metabolism unit.

Collection and Cleaning

Because this detailed procedure is quite lengthy, it appears in Appendix III for the interested reader. There are several adaptations to our procedure which might be just as acceptable, depending upon the degree of solubility of the material, but the one presented does meet the

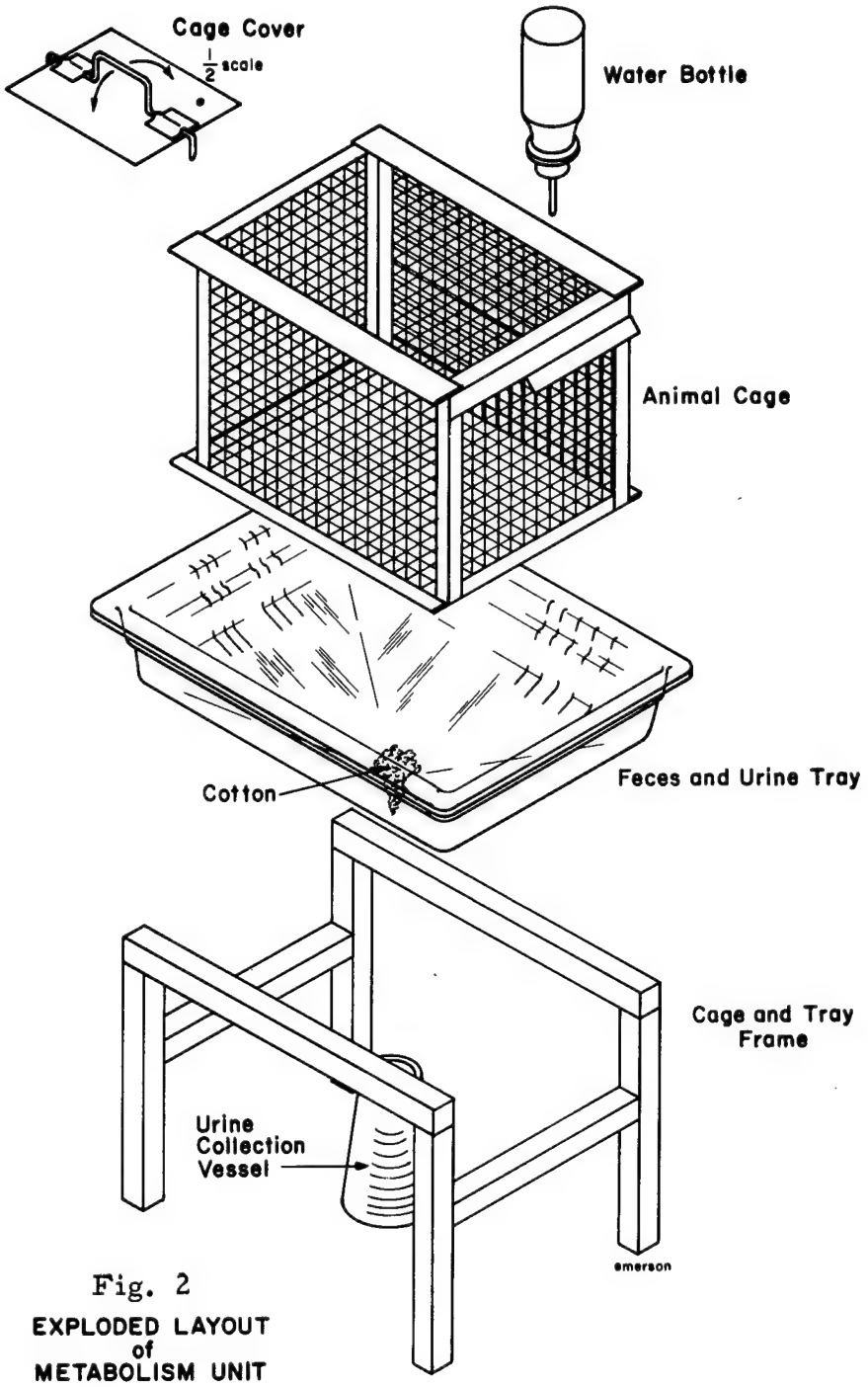


Fig. 2
EXPLODED LAYOUT of METABOLISM UNIT

general requirements of sample collection set forth above.

WHOLE BODY COUNTING

General Procedure. In order to express the daily excreta (radioactivity contained therein) in terms of body content on the particular day of collection, it is necessary to obtain this latter value in some convenient manner. Without whole body counting techniques the usual procedure is to summate all excreta following the day of interest and add the result to the body content at day of sacrifice. This is a cumbersome process, particularly when radioactive decay is involved, and can also lead to a summation of any errors inherent in daily excretion analyses. Thus, if daily (or periodic) whole body counting can be accomplished accurately, a great number of problems can be circumvented.

In many studies, it is only desirous to obtain data on the kinetics of elimination from the whole body and the individual tissues. In that instance, whole body counts are obtained without thought to whether the animals excrete during the process of being handled, or during their residence time in the counter. However, in our studies the procedure is dictated by the philosophy of wanting determinations on excreta, as just discussed, and therefore a counting technique is needed which does not lead to a loss of either urine or feces in the process. The obvious way to accomplish this is by a whole body radioactivity determination directly from the animals as they remain in the cage. A cage count would, therefore, yield a value for the numbers of animals housed therein (one guinea pig, two rats, etc.) which, in turn would match the daily composite excretion data for that cage.

The Detection System. To accomplish the above goals, a counter consisting of twin crystals between which the cage could be inserted was devised. Two 3" x 5" diameter NaI crystals were placed horizontally, facing each other, at a separation distance of approximately 10 inches. Two tracks were then placed between the crystals at the exact separation which allowed them to serve as runners upon which to slide the cage and

its metabolism tray. A drawing is shown in Figure 3.

If the object to be counted were to remain in one position during a determination, then the geometry (and efficiency) would remain constant with time. However, with freely moving animals this is not the case and a form of restraint was devised to hold them in a reasonably constant geometry position. This is accomplished by placing two rats in a plastic container with the bottom removed so that any excreta produced during the procedure falls through the cage bottom and is collected in the standard manner. The counting is done at the time the animals are transferred to the clean cage; any urinary excretion taking place during the brief counting interval does not have sufficient time to soak through the cotton.

Output of each of the two pre-amplifiers (one on each crystal) is fed through an isolation diode to a common lead and the composite counting pulses are fed into one scaler. Using this setup, a result is obtained for the cage count with minimal geometry sensitivity and a maximized counting efficiency ($\approx$ 6% overall). Most of the shielding is supplied by four inches of lead around the counter.

Future Whole Body Counting Schemes. To date, the quantities of radioactivity dealt with have been at the tracer level and the system mentioned above has proven quite satisfactory. However, the long-range plans of the Program encompass studies at very high levels of γ-emitting isotopes as well. This requires a system which will detect activities up to several millicuries per animal, but deliver counting rates within the range covered by ordinary scaling systems. Also, several isotopes will eventually be used in mixtures and a spectral energy separation will be required. Thus, a detection system capable of good energy separation, such as NaI crystals, as opposed to liquid or plastic scintillators, will be used.

The new counting setup will have two NaI crystals such as the ones now in use. They will be mounted in a similar manner, but will be attached to a worm gear arrangement enabling them to be separated by various equal distances from the cage. For very strong sources, each

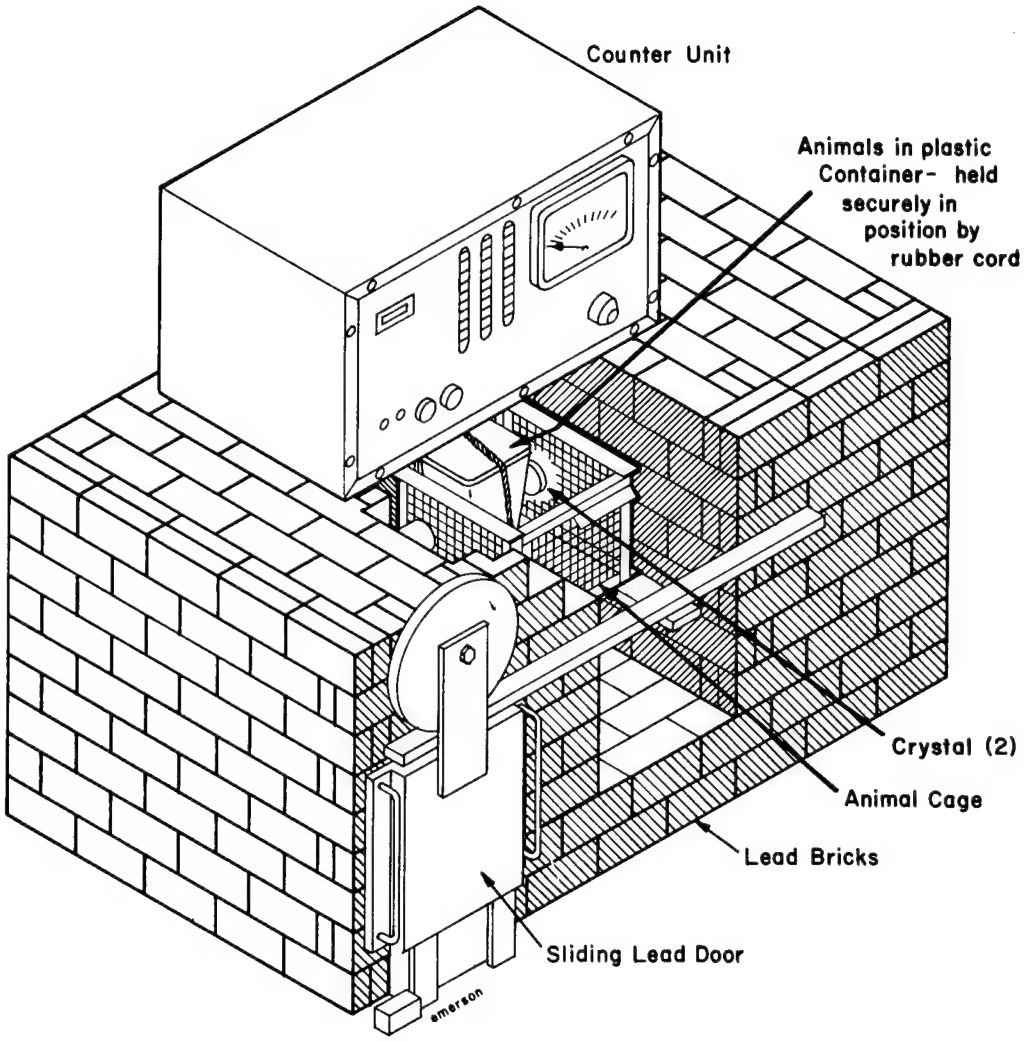


Fig. 3

DUO CRYSTAL WHOLE BODY COUNTER

with brick-breakaway at top to show positioning of animal cage for measuring

crystal will be moved to a distance of at least one meter from the cage, enabling a substantial reduction in the counting rate. As a given cage of animals loses its initially deposited activity, either by physical or biological decay, the crystals will be moved closer at precisely calibrated steps until the levels are so low that they are not significantly detectable at the closest distance. Thereafter, when the animals are being followed for the very long retention component, they will be counted with a more sensitive liquid scintillation system. Although this latter will not give long term spectral distribution, by that time the number of original isotopes remaining in the body should be reduced considerably.

TISSUE PROCUREMENT

Sacrifice Procedures

1. Past and Present Techniques

Sacrifices to date have been accomplished by "maximum" blood withdrawal from the heart. This is done under ether anesthesia utilizing a number 20 gauge needle and ordinary syringe. For a 200 gram rat the average amount of blood obtained varies from 4 to 8 cc's.

2. Possible Future Techniques

Heart puncturing is a particularly bad sacrifice technique for animals which have been exposed by the inhalation route. Occasionally a lung puncture will occur and almost always blood will appear somewhere in the thoracic cavity due to seepage from the wound. The work to date, on cesium chloride, has not presented a problem in cross-contamination because of its rapid loss from lung and blood due to its extreme solubility. However, this could create a sizeable problem in data analysis with other elements and/or compounds introduced into the lung.

For the "immediate" sacrifices used for obtaining deposition data, it is hoped that some sudden and non-traumatic procedure can be used. Electrocution appears to offer one solution to this, being both "clean" and fast, and has the only outstanding disadvantage of being a

potential personnel hazard. For serial sacrifices in the distribution and excretion studies, a method will be used which will be fast and uncomplicated, such as electrocution, or a gaseous death, e.g., chloroform or nitrogen, for those animals upon which no blood sampling seems necessary. Only those animals which have important amounts of circulating isotope, as determined from corollary studies, will be sacrificed by a blood withdrawal technique. Animals used for the "clinical" studies and from which blood is a necessary tissue will be sacrificed in a manner set forth by the investigator desiring the sample.

3. Pre-dissection Procedures

Following sacrifice, the animals are bagged (polyethylene) and placed into a deep freeze. When necrotomy is to take place the animal is removed the previous evening and allowed to thaw overnight in a refrigerator. By following these procedures, the animals have very little time for massive autolysis.

General Necrotomy Practices. The general philosophy underlying good dissection procedures in this type of work is very straightforward. It involves obtaining tissues or samples from the animal without cross-contamination of any origin. In practice, the methods can be likened to work with bacteriological and virological media. In addition to cross-contamination there is an added problem in the Program which involves radiation hazards to the personnel performing the dissections. A few animals at sacrifice or death will contain large amounts of γ-emitting isotopes and the usual procedures will not be allowed. These dissections will probably be done in parts: the animal will be hurriedly severed into well defined sections, each of which represents a lower than tolerable amount of radiation. Also, shielding will be provided wherever it is feasible. There is also the added difficulty, in dissecting the animals for pathology, of removing the tissue sections before autolysis occurs, but at the same time adhering to the anti-contamination procedures. In these studies, only the remainder of the organ (whole organ minus section for pathology) will be weighed and analyzed for its radioactivity,

giving the necessary data for dosage calculation.

Some of the most common sources of contamination are listed below:

1) At any time post-exposure, particularly at early sacrifice times, the pelt may be a source of contamination of the muscle underlying the skin, of the operator's gloves and instruments, of the working surface and possibly of internal organs. This applies mostly to the hair and skin of the head. Following removal of the pelt, the aforementioned muscle, gloves, instruments and working surface should be rinsed and/or wiped with a damp sponge.

2) If the inhaled material is soluble in body fluids, blood may become an important contaminant. A time lapse of several hours between sacrifice and dissection (when practical) will prevent much of this. However, it is still advisable to remove any pools of blood which form during the dissection, using a gauze sponge, and to "blot" any particularly bloody organs or tissues prior to placement in plastic bags for weighing and counting.

3) Of the major internal organs, lung is removed last as it may be assumed to contain a large portion of the inhaled material. It is probable that cross-contamination would exist should material pass out of the trachea or should the lung tissue be cut upon removal. This is avoided by not touching the respiratory tract until the other organs have been excised.

4) The gastrointestinal (GI) tract may be expected to contain large portions of inhaled material due to ciliary action in the respiratory tract which carries material upwards to be swallowed. Of the organs in the peritoneal cavity, the GI tract is removed last, just before the thoracic cavity is entered. Any necessary separation of portions of the GI

tract, as well as removal of its contents, should be done outside of the body.

Specific Organs and Tissues. Appendix IV describes some of the general dissection procedures and lists materials required and organs and tissues of interest.

APPENDIX I

Manufacture of Pneumatic Collars

1. The Collar Mold (see figure 4)

The only materials needed are a one-fourth inch lucite sheet and a one-eighth inch lucite rod. A 3.5 x 3.5 inch square is cut from the sheet and a round hold is drilled completely through, at the center. The diameter of the hole depends upon the size of the animal's head and of the neck for which the mold is to be used. It is polished and rounded very smoothly at the inside edges. Approximately 3/16" from the rim of this hole, and at no particular place on the plate, a hole is drilled within which to insert a section of the lucite rod, so that it protrudes above the surface by about two inches. The rod is fixed in place by a suitable glue.

2. The Collar

A liquid latex, LOTOL (Naugatuck Chemical Division, U. S. Rubber Co., Naugatuck, Conn.), is used to construct the pneumatic collars. This material is soluble in distilled demineralized water and dilute ammonia and, hence, can be diluted to any desirable consistency. The LOTOL is painted on the mold, dried under infra-red, repainted, dried, etc., until the desired thickness of coat is attained. Other more efficient and automated methods could be perfected for doing this, but the personal observation during application does have certain advantages. For one thing, it can help eliminate weak spots which might occur from other application techniques such as spraying.

When the final coat is dried, the collar is then loosened from the mold around all four of the peripheral edges. The latex is then peeled away from both top and bottom with talc being applied concurrently to all exposed surfaces. The last step in removal is peeling the latex from the lucite rod. The collar is then turned right side out and is ready for use.

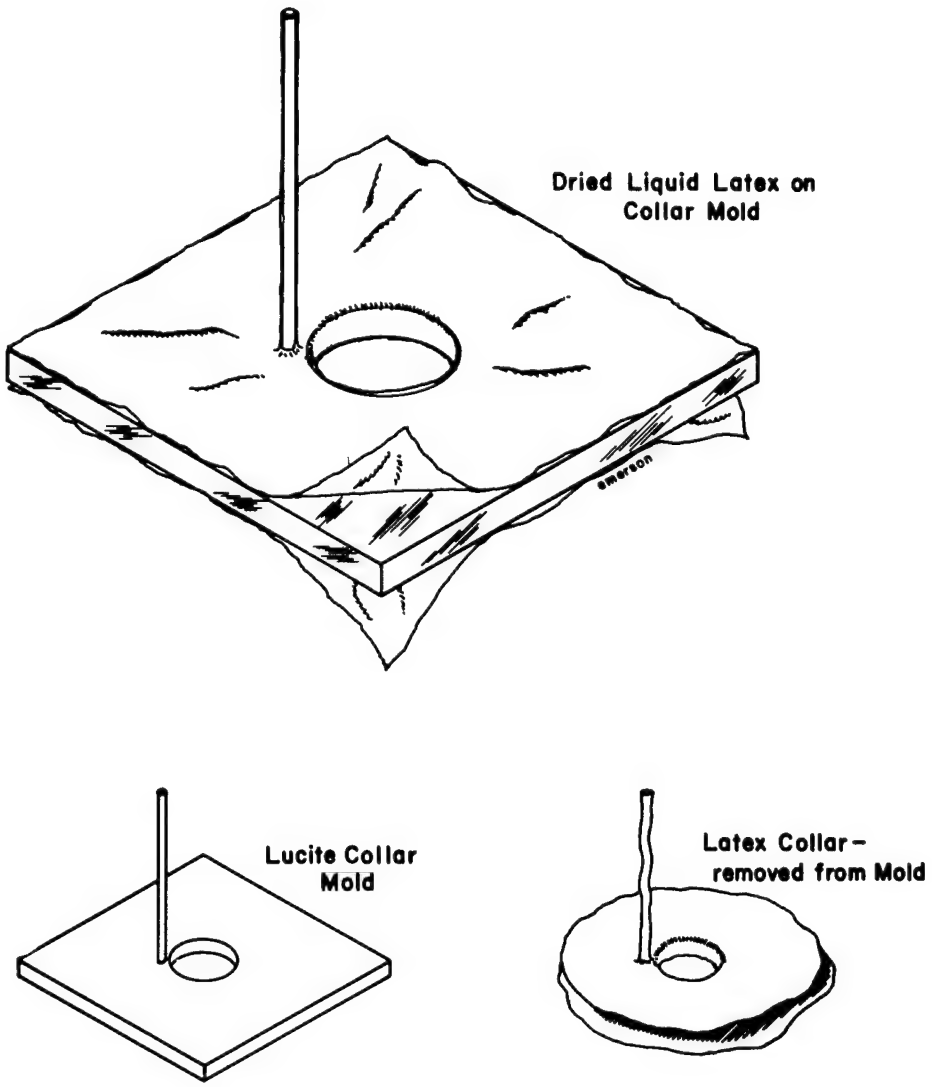
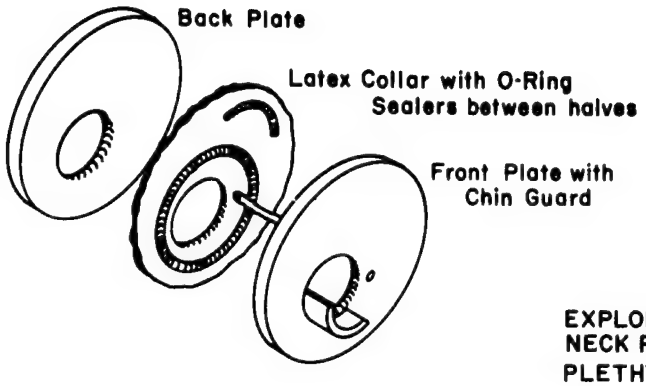


Fig. 4

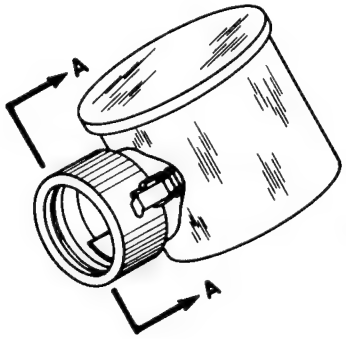
COLLAR MOLD and LATEX COLLAR

3. The Collar Attachment

Two lucite plates are cut to fit the part of the plethysmograph which will house the animal's neck (See Figure 5). With guinea pigs it is advantageous to fit the collar about the forehead and lower mandible because of the disproportionate size of the head and neck. A hole just large enough to comfortably permit the passage of the animal's head is bored in each plate, somewhat off-center, toward what will ultimately be the bottom (with the ventral side of the animal). Very near to this opening, in one plate, and near the top of one side is a hole just large enough to permit passage of the "stem" of the collar, or, that part which was removed from the lucite rod. A small lucite trough protrudes from one plate so that the animal's chin (head) rests on this when it is inserted. An "O" ring of proper dimensions is slipped over the collar and centered about the opening, but allowing the latex stem opening to be inside the ring. (See diagram) The outer edges of the collar are trimmed to a circle of proper size, the two plates are squeezed toward each other giving a tight seal between the outer plate, outer side of the collar, "O" ring, inside of the collar, and outside of inner plate. The entire setup is seated into the plethysmograph at the proper place.



EXPLODED LAYOUT of
NECK PLATES for
PLETHYSMOGRAPHS



PLETHYSMOGRAPH

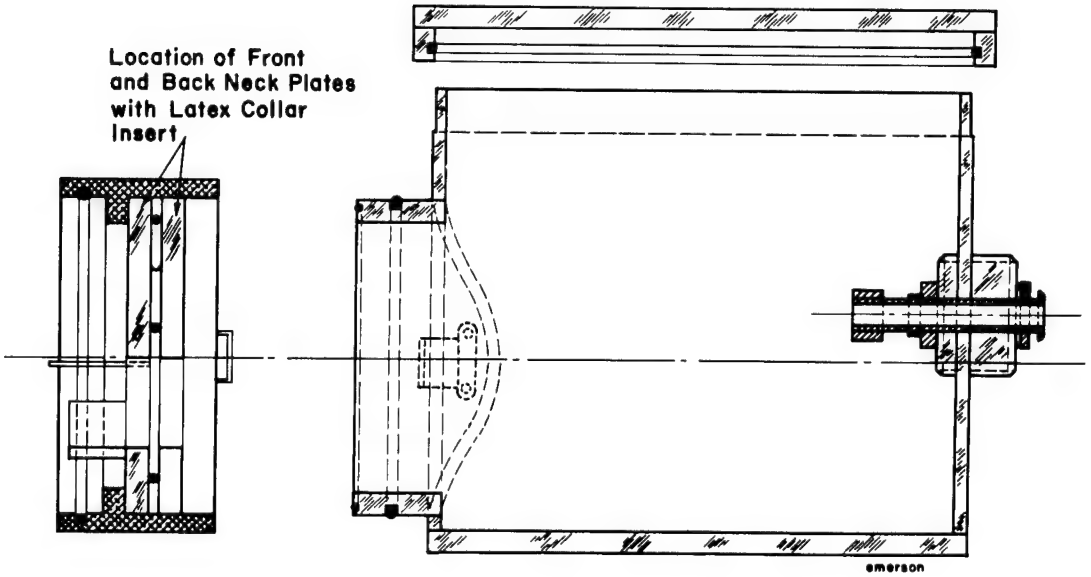


Fig. 5 EXPLODED LAYOUT · SECTION "A–A"

APPENDIX II

Calculation of Proper Plethysmograph Volume

The standard gas law pertaining to small changes in pressure inflicted by a small change in volume is expressed as,

$$(1)\quad \frac{\Delta V}{\Delta P} = \frac{V}{P}$$

where, in the case of a plethysmograph,

V = minimum volume allowable

ΔV = change in volume due to breathing

P = ambient pressure (613 mm Hg)

ΔP = overpressure or maximum allowable change (assumed to be approximately 1 cm H_2O = 0.737 mm Hg)

If it is assumed that the largest guinea pig to be exposed is 350 grams and that its tidal volume is 2.5 cc, a usable plethysmograph volume, V_P, can be calculated.

$$(2)\quad V_P = 350 + \frac{P\Delta V}{\Delta P} = 350 + \frac{(613)\ (2.5)}{0.737} = 2429 \text{ cc.},$$

assuming that the density of the guinea pig is 1.0.

On the basis of the above assumptions and computations, the plethysmographs were constructed to approximate this volume.

APPENDIX III

Detailed Procedures for Collection of Urine and Feces

1. Materials

   a. The Metabolism Unit (See Figure 2)

      Wooden stand
      Plastic tray
      Cotton
      Screen
      Cage
      Cage top
      Water bottle
      Urine container

   b. Cage Cleaning

      Gauze sponges
      Forceps
      Plastic spoon
      Polyethylene water bottle
      Warm water
      Pill boxes
      Masking tape
      Pen or marking pencil
      Gloves

2. Procedures

   a. General Rules

(1) Excessive use of water in cage cleaning is not generally warranted. In rinsing the feces, in particular, the use of too much may remove fecal material as well as urine, washing it down into the urine container. Following removal of feces from the screen, less caution need be taken as to the amount of water used, but the level must never exceed a certain specified point below the top of the container. As the

entire cage cleaning may not fill the container to that point, additional water should be added after cleaning has been completed. This requirement is dictated by a necessity for constant counting geometry.

(2) A minimal number of sponges should be utilized while still accomplishing optimum cleaning. Unless otherwise instructed, sponges used in removing food are treated like those for absorbing urine and should be placed with the urine sample.

b. Step by Step Directions

Because of the time involved between the first steps of the cage cleaning procedure, it is efficient to work on from three to five cages at a time, as long as rinse water does not evaporate before being wiped off.

(1) Remove any pieces of food remaining on the cage floor and, unless there is a great deal of fecal material on the food, it should be placed in the urine container. If this is not immediately feasible, place the food on a gauze sponge or paper towel until the cage cleaning is complete. Food which is particularly contaminated with feces should be placed in the fece container. Next, rinse the inside of the cage and the feces on the screen beneath, leaving the cage in place on the plastic tray.

(2) Wipe the inside and bottom. Set the cage on towelling or Kimpac (Kimberly-Clark, Inc.) on a utility cart. Never place a cage on an uncovered surface. Next, using a spoon, scoop the feces toward the center of the screen, lift the screen and check to see if there are feces in the tray. If so, pick them out and place them on the screen. Then, holding a gauze sponge with forceps, wipe the underside of the screen, thus drawing off water from the first rinsing. Replace the screen (plus feces) in the tray and rinse again with warm water. Set the used sponge on one edge of the screen.

(3) Lift the screen and, using the gauze sponge, wipe the underside of the screen. Repeat this on each of the three to five cages.

At this point, each cage will have been wiped clean and the screens rinsed and wiped twice. Using the spoon, scoop the feces up and place in the labeled pill box provided for the metabolism unit being cleaned.

When all of the fecal material has been removed from the unit, the screen should be rinsed down, into the plastic tray, holding the screen vertically and rinsing both sides with warm water. Then the screen should be wiped with a gauze sponge on both sides. This procedure must be repeated until the screen is clean. Then the screen may be placed next to the metabolism unit on a paper towel. The sponge, or sponges, used is placed in the urine container following the collection.

(4) Food and urine are scraped down towards the center of the tray with a spoon. The cotton plugging the hole in the tray is pulled through and into the urine container, allowing wash water, urine and food to pass through into the container. The tray is then to be rinsed down and wiped with a gauze sponge, and the procedure repeated as much as necessary to clean the tray completely. Remove the urine container and add the large pieces of food and any sponges which have been laid aside until this point.

APPENDIX IV

Dissection Techniques

1. Materials and Preliminary Procedures

a. In preparation for a dissection, the working surface is covered with a polyethylene sheet, taped down along the edges. Clean instruments are placed on the sheet, in an easily accessible position. An instrument kit for small animal dissection consists of the following instruments:

1 blade forceps
4 No. 3 knife handles
1 No. 15 blade
2 No. 10 blades
2 No. 12 blades
1 probe point, dissecting scissors
1 straight, fine point forceps
2 blunt dissecting forceps
1 mouse tooth tissue forceps
1 heavy duty dissecting scissors
1 micro dissecting, fine sharp scissors
3 straight hemostatic forceps
1 pair surgeons gloves

Pre-weighed and numbered bags for tissues are folded (the tops are folded over to prevent contamination of the outside of the bag) and taped in an accessible position above the working bench.

b. After removal from the refrigerator, the animal is whole body counted and weighed, and the body weight, blood volume, date of sacrifice, day of experiment and animal identification are recorded on the weighing sheet prior to beginning dissection. This is extremely important as it will be the only record of most of these data once the bag in which the animal is wrapped has been discarded.

2. Dissection Technique - General Comments

a. Many organs and tissues of the body are delicate and should not be grasped firmly with forceps or hemostat while being removed. It is nearly always possible to hold an organ securely by grasping the nearest visible connective tissue or a connecting vein or artery. Exceptions to this are the exorbital lacrimal salivary glands, trachea, esophagus, uterus and bladder which may of necessity be held directly with forceps. Blunt forceps should be used for this procedure and care should be taken.

b. As much as possible, dissections should be performed in a "semi-blunt" manner. Where a scalpel is not necessary, or where its use may injure an important tissue, such use should be avoided. It is most desirable to leave organs intact as a preventive measure against cross-contamination.

c. In cutting, the sharp edge of the scalpel blade need not, in most instances, come into direct contact with the tissue which is being removed. Although organs or tissues appear to be closely connected to one another, some tension applied between two organs will show that they are not. It is the connective tissue between two organs which should be cut. Assuming that most organs are being held by the closely allied connective tissue, cutting is done against the forceps or hemostat rather than against the tissue.

d. If feasible, the dissection should not be performed in a direct draft as this tends to disseminate loose hair and to dry the tissue, thereby changing the tissue weight.

3. Organ and Tissue Samples

The following is a list of the samples removed from the small animals for analysis of their radioactivity:

Hair
Head skin
Skin

Salivary glands
Spleen
Adrenal glands
Kidneys
Liver
Esophagus
Stomach
Stomach contents
Small intestine
Small intestine contents
Large intestine
Large intestine contents
Mesentery
Abdominal lymph nodes
Ovaries/testes
Uterus/seminal vessicles
Bladder
Fat
Diaphragm
Thyroid
Thymus
Trachea
Bronchi
Bronchial lymph nodes
Mediastinum
Lung - R. apical
Lung - R. cardiac
Lung - R. diaphragmatic
Lung - R. intermediate
Lung - L. apical - cardiac
Heart
Nares
Brain

Head
Teeth
Bones
Femur (whole)
Femur (split)
Femur marrow
Spinal cord
Muscle
Paws and tail
Blood
Miscellaneous
Carcass

4. Preparation for Counting

The bag plus contents are weighed at completion of the dissection and the top of the bag is then sealed permanently with a hot iron. Only on very rare occasions has there been a detectable leakage of fluid. The bags are then frozen and stored until time for counting. The tissues are counted in the frozen or semi-frozen state, depending upon the length of residence at room temperature in the counting room.

A report describing the counting techniques and equipment, along with general data processing procedures, is being written and will appear as an LF report in the near future.

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